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In Re Application of: Frank Grosveld **Confirmation No.: 1498**

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For: U.S. Continuation Patent Application Entitled, "IMMUNOGLOBULIN 2"

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DATE OF DEPOSIT: September 26, 2006

Commissioner for Patents
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Dear Sir:

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Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country: Great Britain

Application No.: GB 0110029.6

Filing Date: 04 April 2001

Applicant respectfully requests that receipt of the enclosed document be acknowledged and that the U.S. Patent Office's records are updated accordingly.

Respectfully submitted,

Dated: September 26, 2006

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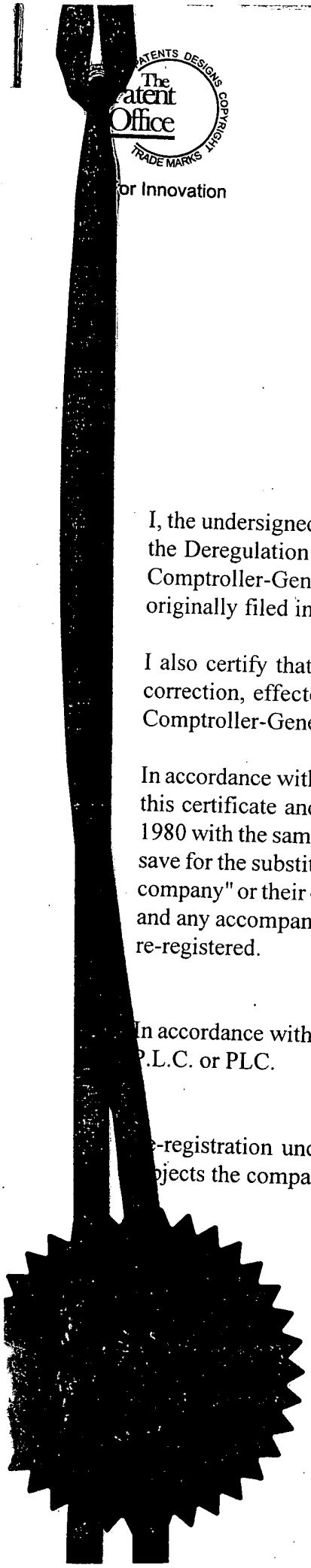

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1. Your reference — P011403GB ATM

2. Patent application number *(The Patent Office will fill in this part)* — **0110029.6**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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AC 4/77 14/4/02

Patents ADP number *(if you know it)*
00434A1002

785962002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — Transgenic animal

5. Name of your agent *(if you have one)* — **D YOUNG & CO**

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) — **21 NEW FETTER LANE**
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Agents for the Applicants

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Transgenic animal

Field of the Invention

The present invention relates to novel transgenic animals expressing a humanised single chain immunoglobulin locus, vectors containing the same and single chain antibodies obtained from transgenic animals.

Background Art

Conventional antibodies are protein complexes that contain heavy and light chains. The heavy chain is coded for by the heavy chain locus while the light chains (κ or λ) are coded for on separate chromosomal loci.

The heavy chain locus contains a large number of variable chain genes (V_H; in fact not complete genes but comprising a first coding exon plus transcriptional start site) that are recombined onto two short coding regions D and J (known as VDJ recombination) which precede the exons that code for the constant region of the heavy chain Cμ to give a complete antibody heavy chain gene known as IgM. Subsequently a class switch takes place where the variable part is recombined with another constant region that is located downstream of the IgM constant region to give IgD, IgG, IgA and IgE (coded for by the exons of the various Cδ, Cγ, Cα, Cε located downstream of the exons for Cμ). The intervening constant regions are deleted in the process. A similar process takes place in the light gene loci, first the κ locus, and when this does not lead to a productive antibody in the λ locus (for review see Rajewski, K., Nature 381, p751-758, 1996; for an extensive review, see the textbook Immunobiology, Janeway, C., Travers, P., Walport, M., Capra. J., Current Biology Publications/Churchill Livingstone/Garland Publishing, fourth edition, 1999, ISBN 0-8153-3217-3).

Camelids (camels and llamas) contain, in addition to normal heavy and light chain antibodies (2 light chains and 2 heavy chains in one antibody), single chain antibodies (containing only heavy chains). These are coded for by a distinct set of V_H segments referred to as V_{HH} genes. Antigen binding for single chain antibodies is different from that seen with conventional antibodies, but high affinity is achieved the same way, i.e. through hypermutation of the variable region and selection of the cells expressing such high affinity antibodies.

At present, single chain antibodies to a specific antigen can be obtained by either:

1) immunising camels or llamas with a specific antigen which produces a set of antibody producing cells in the blood which express different antibodies with different specificities and affinities; or

2) screening an artificially constructed camel or llama antibody library.

Option 1) has the disadvantage that it is generally inconvenient as it requires the use of camels or llamas and is only able to produce camel and llama derived antibodies and whilst option 2) does not require the use of camelids, the antibodies are still limited those of camelid origin and the range of possible antibodies generated is restricted by the original in vitro synthesis of the DNA coding for the variable region. In addition in vivo selection for high affinity antibodies cannot be carried out.

The reduction of host immunogenicity of artificial antibodies produced by conventional means is essential if the antibodies are destined for human therapeutic applications. The generation of chimeric and humanised antibodies, for example as set forth in European patent 0239400, has succeeded in reducing the immunogenicity of monoclonal immunoglobulins to a great extent. However, humanised antibodies are produced from artificial libraries, which suffer from the same drawbacks as mentioned for 2) above.

The expression of heterologous immunoglobulin loci in mice is known. For example WO00/26373 describes the expression of a human Igλ locus in a murine model.

Summary of the Invention

In a first aspect, the present invention relates to a transgenic non-human mammal which expresses a heterologous camelid heavy chain immunoglobulin locus.

Advantageously, the endogenous heavy chain locus is deleted and replaced with the camelid locus. The camelid locus may consist entirely of camelid sequences; in a preferred embodiment, however, the camelid locus comprises variable domain genes of camelid origin, and heavy chain constant domain genes of human origin.

The transgenic animal is advantageously smaller than a camelid and easier to maintain and immunise with desired antigens. Ideally, the transgenic animal is a rodent, such as a

rabbit, guinea pig, rat or mouse. Mice are especially preferred. Alternative animals, including goats, sheep, cats, dogs and other domestic or wild mammals, may also be employed.

Advantageously, the camelid locus comprises:

(i) a first locus including a camelid V_{HH} region, a D region and a J region capable of recombining to form a VDJ coding sequence encoding a variable heavy chain polypeptide including a complete antigen binding site; and

(ii) a second locus including at least one exon encoding a constant heavy chain polypeptide

Advantageously, the first locus comprises sequences of camelid origin; the J and D regions, though may be of other mammalian, including human, origin.

As set forth above, camelids contain a distinct set of V_H segments referred to as V_{HH} genes. The V_H and V_{HH} appear in the same functional region of the genome (i.e. they appear mixed in between each other in the dromedary genome). The identification of an identical D segment in a V_H and V_{HH} cDNA suggests the common use of the D segment for V_H and V_{HH}. Natural V_{HH} containing antibodies are missing the entire C_H1 domain of the constant region of the heavy chain. The exon coding for the C_H1 domain is present in the genome but is spliced out due to the loss of a functional splice acceptor sequence at the 5'side of the $C_H1$exon. As a result the VDJ region is spliced onto the C_H2 exon. When a V_{HH} is recombined onto such constant regions ($C\gamma2$, $C\gamma3$) an antibody is produced that acts as a single chain antibody (i.e. an antibody of two heavy chains without a light chain interaction). Binding of an antigen is different from that seen with a conventional antibody, but high affinity is achieved the same way, i.e. through hypermutation of the variable region and selection of the cells expressing such high affinity antibodies. The same process of hypermutation seen in camelids is replicated in the transgenic animals according to the invention.

The second locus may be of human or other mammalian origin, as well as of camelid origin. As set forth above, the C_H1 exon is preferably incapable of being expressed, or has a splice regulatory region disabled, such that the VDJ region is spliced directly to C_H2-C_H3.

Thus, the present invention also provides a transgenic animal comprising:

(i) a first locus including a V_{HH} region, a D region and a J region capable of recombining to form a VDJ coding sequence encoding a variable heavy chain polypeptide including a complete antigen binding site; and

(i) a second locus including at least one exon encoding a constant heavy chain polypeptide;

wherein, the transgenic animal is incapable of producing immunoglobulin with light chains, and upon insertion, the first and second loci are expressible and their respective coding sequences are capable of recombination to encode a complete heavy polypeptide chain.

The invention moreover provides a vector for producing a transgenic animal in accordance with the above aspect. Such a vector may be any suitable vector, but vectors capable of inserting large amounts of nucleic acid, sufficient to encode an entire immunoglobulin heavy chain locus, are preferred. Such vectors include artificial chromosomes, such as YACs. Endogenous mouse heavy chain loci are advantageously silenced or deleted, for example as described in WO00/26373 or WO96/33266; see also Li and Baker, (2000) Genetics 156(2):809-821 and Kitamura and Rajewsky, (1992) Nature 356:154-156.

Antibody-producing cells may be derived from transgenic animals according to the invention, and used for example in the preparation of hybridomas for the production of single chain antibodies. Moreover, nucleic acid sequences may be isolated from transgenic animals according to the invention an used to produce single chain antibodies using recombinant DNA technology, as is known in the art.

Furthermore, the present invention provides a method of producing a transgenic animal capable of producing immunoglobulin lacking light polypeptide chains and comprising two heavy polypeptide chains the method comprising inserting into the genome of the animal:

(i) a first locus including a V_{HH} region, a D region and a J region capable of recombining to form a VDJ coding sequence encoding a variable heavy chain polypeptide including a complete antigen binding site; and

(ii) a second locus including at least one exon encoding a constant heavy chain polypeptide;

wherein, upon insertion, the first and second loci are expressible and their respective coding sequences are capable of recombination to encode a complete heavy polypeptide chain.

The invention moreover provides methods for producing single chain antibodies comprising immunising a transgenic animal as described in the preceding aspects of the invention, and antibodies produced by such techniques. Antibodies according to the invention are useful in a variety of applications, including reagents, diagnostics and therapeutics – and including any application in which the small size and enhanced stability of single chain antibodies are advantageous.

Brief Description of the Drawings

Figure 1 is a schematic summary of the assembly of a construct according to a preferred embodiment of the present invention; and

Figure 2 is a schematic illustration of a construct according to a preferred embodiment of the present invention.

Figure 3 is a schematic illustration of an isolated nucleic acid construct according to the invention.

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. - and the full version entitled Current Protocols in Molecular Biology, which are incorporated herein by reference) and chemical methods.

Transgenic Loci

As set forth above, transgenic loci according to the invention comprise a V_{HH}-D-J locus and a C_H locus. Preferably, the coding sequences are from camelids and another mammal, such as human. When the coding sequences include human sequences, transgenic animals including the construct are adapted to produce humanised antibodies in response to antigen challenge.

Preferably, the V_{HH} region of the first locus comprises at least one V_{HH} exon. When the V_{HH} region comprises a plurality of V_{HH} exons it may include at least 2, at least 5 or at least 10 V_{HH} exons. Preferably, it includes the entire camelid V_{HH} locus.

For the purposes of the present invention the term "V_{HH} exon" includes naturally occurring V_{HH} coding sequences such as those found in camelids and derivatives of naturally occurring V_{HH} coding sequences that include any substitution of, variation of, modification of, repiacement of, deletion of or addition of one (or more) nucleic acids from or to the sequence of a naturally occurring V_{HH} coding sequence, provided the resultant nucleotide sequence is able to form a VDJ exon encoding a functional variable heavy chain polypeptide.

Naturally occurring V_{HH} exons may be obtained from a variety of sources readily apparent to one skilled in the art including camelid genomic DNA, cDNA, PAC, BAC or YAC libraries. Alternatively, the V_{HH} exons may be chemically synthesised using established techniques and the available nucleic acid sequence information.

Preferably, the D region of the first locus comprises at least one D exon. The D exon may correspond to a naturally occurring sequence and preferably is of human or camelid origin, however, the D exon may be from other mammals. In this regard, the particular choice of D exon will depend at least partly on the intended use of the construct. For example, if the construct is to be used to produce a transgenic animal, the choice of the D exon may depend upon the intended use of antibodies produced therefrom. In this regard, if it is desired to produce humanised antibodies then the D exon may be of human origin.

When the D region comprises a plurality of D exons it may include at least 2, at least 5 or at least 10 D exons. Preferably, it includes an entire D locus.

Preferably, the J region of the first locus comprises at least one J exon. The J exon may correspond to a naturally occurring sequence and preferably is of human or camelid origin, however, the J exon may be from other mammals. In this regard, the particular choice of J exon will depend at least partly on the intended use of the construct. For example, if the construct is to be used to produce a transgenic animal, the choice of the J exon may depend upon the intended use of antibodies produced therefrom. In this regard, if it is desired to produce humanised antibodies then the J exon may be of human origin.

As was the case for "V_{HH} exon", for the purposes of the present invention the terms "D exon" and "J exon" includes naturally occurring D and J exons such as those found in humans and other mammals and derivatives of naturally occurring exons that include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acids from or to the sequence of a naturally occurring sequence, provided the resultant nucleotide sequence is able to form a VDJ coding sequence encoding a functional variable heavy chain polypeptide.

Naturally occurring D and J exons may be obtained from a variety of sources readily apparent to one skilled in the art including genomic DNA, cDNA, PAC, BAC or YAC libraries. Alternatively, the D and J exons may be chemically synthesised using established techniques and the available nucleic acid sequence information.

Preferably, the exon included in the second locus is one or more exons selected from the group of exons encoding a constant heavy chain ("C_H exons") comprising: Cμ, Cδ, $C\gamma_{1-4}$, Cε and $C\alpha_{1-2}$. Even more preferably, the second locus includes a Cμ exon and at least one other C_H exon. In one particular form of the invention the second locus includes a Cμ exon, a Cδ exon and at least one other C_H exon.

As was the case with the exons discussed above, for the purposes of the present invention the terms "C_H exon(s)" includes naturally occurring C_H exons such as those found in humans or camelids and other mammals and derivatives of naturally occurring exons that include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acids from or to the sequence of a naturally occurring sequence, provided the resultant nucleotide sequence is capable of recombination with the VDJ coding sequence in the first locus to encode a functional complete heavy polypeptide chain.

Generally C_H exons include 3 or 4 domains (C_H1-C_H4) that encode different portions of the constant heavy chain polypeptide. According to the present invention one or more of the C_H exon(s) in the second locus may be modified to render them incapable of expressing the C_H1 portion of their encoded polypeptide. Preferably, the C_H1 domain of the C_H exon(s) are modified to remove a splice acceptor site 5' of the C_H1 domain. The splice acceptor site may be removed by modifying its nucleotide sequence or removing portions thereof to render it non-functional. Alternatively, the C_H1 domain may be rendered non-functional by, for example, targeted gene deletion of the entire domain.

Modifying the C_H1 domain facilitates the recombination of the VDJ coding sequence with the C_H2 domain of respective C_H exons. Preferably, all the C_H exons included in the second locus are modified to render them incapable of expressing their C_H1 domain. However, in instances where switching and/or antibody maturation cannot take place in the absence of at least one C_H exon capable of expressing its C_H1 domain then the second locus will include at least one C_H exon including a functional C_H1 domain. When at least one functional C_H1 domain is required it is preferably the C_H1 domain of Cμ and/or Cδ exon.

The first and second loci may include additional sequences that affect their transcription and post transcriptional modification. Such additional sequences include regulatory regions required for expression. In this regard, preferably, the second locus further comprises a regulatory region such as an immunoglobulin heavy chain LCR (Gram, H., G. Zenke, S. Geisse, B. Kleuser, and K. Burki (1992) Eur. J. Immunol. 22:1185-1191). The regulatory regions may be of mammalian origin and preferably are of human origin.

The constructs of the present invention may be produced using standard techniques. In particular, the various coding sequences in the construct may be obtained as recombinant DNA from a PAC (P1-derived artificial chromosome), BAC (bacterial artificial chromosome) or YAC (yeast artificial chromosome) library or chemically synthesised.

Vectors

The present invention also provides vectors including a construct of the present invention. Essentially two types of vectors are provided, replication vectors and transformation vectors.

A. Replication

Constructs of the invention can be incorporated into a recombinant replicable vector such as a BAC vector. The vector may be used to replicate the construct in a compatible host cell. Thus, in a further embodiment, the invention provides a method of making constructs of the invention by introducing a construct of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the construct. The construct may be recovered from the host cell. Suitable host cells include bacteria such as *E. coli*, yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

B. Transformation

The constructs of the present invention may also be incorporated into a vector capable of inserting the construct into a recipient genome and thus achieving transformation. In addition to the construct of the present invention such transformation vectors may include one or more of the following components.

Promoters

The promoter is typically selected from promoters which are functional in mammalian cells, although prokaryotic promoters and promoters functional in other eukaryotic cells may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as promoters of α-actin, β-actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of immunoglobulin genes). They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the Rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter. It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Tissue-specific enhancers capable of regulating expression in antibody-producing cells are preferred. In particular, the heavy-

chain enhancer required for the successful activation of the antibody gene locus in vivo (Serwe, M., and Sablitzky, F., EMBO J. 12, p2321-2321, 1993) may be included. Locus control regions (LCRs), particularly the immunoglobulin LCR, may also be used. Chimeric promoters may also be used comprising sequence elements from two or more different promoters.

Other Vector Components

In addition to a promoter and the construct, vectors of the present invention preferably contain other elements useful for optimal functioning of the vector in the mammal into which the vector is inserted. These elements are well known to those of ordinary skill in the art, and are described, for example in Sambrook et al., Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, 1989.

Construction of Vectors

Vectors used for transforming mammalian embryos are constructed using methods well known in the art, including, without limitation, the standard techniques of restriction endonuclease digestion, ligation, plasmid and DNA and RNA purification, DNA sequencing, and the like as described, for example in Sambrook, Fritsch, and Maniatis, eds., Molecular Cloning: A Laboratory Manual., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1989]). In general, vector construction will include the following steps:

a) The endogenous mouse locus is inactivated, for example using one of the published knockout procedures (e.g. Kitamura, D and Rajewski K., Nature 352, p154-156, 1992).

b) The DJ and IgM region of the human heavy chain locus is isolated (the camelid locus itself may be used for non humanised antibodies; loci of any other species may be employed to obtain antibodies with corresponding constant regions) as a recombinant DNA from a human PAC, BAC or YAC library and cloned as a restriction enzyme fragment, for instance a Sal1 fragment. This region also contains the heavy chain enhancer required for the successful activation of the antibody gene locus in vivo (see Serwe, M., Sablitzky, F., EMBO J. 12, p2321-2321, 1993).

c) A number of llama V_{HH} genes are first cloned as cosmids through the construction of a llama genomic DNA library by conventional techniques. Since the V_{HH} genes are located

among the regular V_H genes they are subsequently cloned as individual restriction fragments into a combination of several V_{HH} by standard techniques. Thus an array of V_{HH} genes is made. The genes may alternatively be left as a single gene. This array of genes can be isolated as a MluI (or other restriction enzyme) fragment.

d) The 3' human immunoglobulin heavy chain LCR, a regulatory region required for the expression of the locus, is cloned as an SceI restriction fragment.

e) The Cγ3 and Cγ2 exons (to become the single heavy chain only regions) are cloned as a separate restriction fragment (for example, AscI fragments for humanised antibodies). The C_H1 regions of these heavy chain genes are rendered non-functional by homologous recombination in bacteria (Imam et al., (2000) Nucleic Acids Res 2000 Jun 15;28(12):E65) by removing the splice acceptor sequences of the C_H1 exon (Nguyen et al., ibid).

Steps b-e provide the pieces for the new IgH locus (Fig.3) that should take over the function of the inactivated mouse locus described under a). This new locus is put together by cloning each of the fragments in the appropriate order into a BAC vector containing a linker region with all of the restriction sites described above (Fig.1), resulting in a novel locus of approx. 220kb (in the example of three V_{HH} genes) that can be isolated as a NotI restriction fragment and purified away from the vector by standard techniques (salt gradients, CSH laboratory manual). The purified fragment (Fig. 3) is subsequently introduced into fertilised mouse eggs derived from the KO mice described in a) by standard techniques to obtain transgenic mice expressing the new locus.

It is not known yet whether switching and antibody maturation can take place in the absence of any conventional antibodies hence the Cμ and Cδ regions are left intact (such heavy chains can interact with mouse light chains whose coding loci are left intact in the mice described under a)). It is however possible that in the camelids a different heavy chain arrangement is used.

It may for example contain Cμ and Cδ regions that do not contain a C_H1 that allow maturation and selection of B cells expressing heavy chain antibodies only.

Alternatively it could contain one Cμ and/or one Cδ set of exons that contain an "abnormal" 5'splice site which allows partial splicing in of C_H1 and result in a cell that could express a heavy and light chain antibody or a heavy chain only depending on the

type of V region (V_H or V_L respectively) that was recombined onto D and J.

Therefore a second locus may be prepared with all the C_H1 regions modified (i.e. also the $C\mu$ and $C\delta$ loci) to test whether normal antibody switching and maturation can take place in the mouse with just single chain antibodies (Fig.3, middle set of arrows). The latter locus would obviously be preferable as it would provide a mouse with single chain antibodies only.

Transgenic Animals

The constructs of the present invention may be introduced into an animal to produce a transgenic animal. Thus, the present invention also provides a transgenic animal including a construct described herein.

Inserting the loci into the genome of a recipient animal may be achieved using any technique apparent to those skilled in the art, for example, microinjection. Following introduction of nucleic acid into an embryo, the embryo may be incubated in vitro for varying amounts of time, or reimplanted into a surrogate host, or both. In vitro incubation to maturity is within the scope of this invention. One common method is to incubate the embryos in vitro for 1-7 days and then reimplant them into the surrogate host.

Reimplantation is accomplished using standard methods. Usually, the surrogate host is anaesthetised, and the embryos are inserted into the oviduct. The number of embryos implanted into a particular host will vary, but will usually be comparable to the number of offspring the species naturally produces.

Transgenic offspring of the surrogate host may be screened for the presence of the transgene by any suitable method. Screening is often accomplished by Southern or Northern analysis, using a probe that is complementary to at least a portion of the transgene. Western blot analysis using a ligand specific for the antibody encoded by the transgene may be employed as an alternative or additional method for screening. Typically, the tissues or cells believed to express the transgene at the highest levels are tested, although any tissues or cell types may be used for this analysis.

Progeny of the transgenic mammals may be obtained by mating the transgenic mammal with a suitable partner, or by in vitro fertilisation of eggs and/or sperm obtained from the transgenic mammal. Where in vitro fertilisation is used, the fertilised embryo may be

implanted into a surrogate host or incubated in vitro, or both. Where mating is used to produce transgenic progeny, the transgenic mammal may be backcrossed to a parental line. Using either method, the progeny may be evaluated for the presence of the transgene using methods described above, or other appropriate methods.

The animal may be varied provided it is a mammal. Preferably, the animal is a non-human mammal such as a rodent and even more preferably a rat or mouse. In this regard, it is also preferred that the recipient animal is incapable of producing antibodies that include light chains or at the very least has a reduced capacity to produce such antibodies. To achieve this end the recipient animal may be a "knock out" animal that is capable of having one or more of the genes required for the production of antibodies with light chains turned off or suppressed.

By using recipient animals incapable of producing antibodies that include light chains or at the very least with only a reduced capacity to produce such antibodies, the method of the present invention enables the efficient production of large quantities of single chain antibodies and antibody producing cells from a transgenic animal according to the present invention upon challenge with a given antigen.

Antibodies

The transgenic animals of the present invention may be used to produce single chain antibodies. Thus, the present invention also provides a method of producing single chain antibodies comprising administering an antigen to a transgenic animal according to the present invention.

The single chain antibodies produced from transgenic animals of the present invention include polyclonal and monoclonal single chain antibodies and fragments thereof. If polyclonal antibodies are desired, the transgenic animal (e.g., mouse, rabbit, goat, horse, etc.) may be immunised with an antigen and serum from the immunised animal collected and treated according to known procedures. If serum containing polyclonal antibodies contains antibodies to other antigens, the polyclonal antibodies of interest can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art.

Monoclonal antibodies of the present invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by

hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against a given antigen epitope can be screened for various properties; i.e., for isotype and epitope affinity.

Antibodies of the present invention may be useful in diagnosis, and those which are neutralising may be useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the agent against which protection is desired. Techniques for raising anti-idiotype antibodies are known in the art. These anti-idiotype antibodies may also be useful in therapy.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies that retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies (scFv). In the particular context of the present invention, "antibodies" refers to camelid single chain (heavy chain) antibodies.

Antibodies of the invention may also be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

The present invention also provides single chain antibody producing cells isolated from the transgenic animal and hybridomas derived therefrom, to nucleic acids encoding single chain antibodies and fragments thereof and vectors and hosts comprising the same.

Uses

The invention provides transgenic animals from which heavy chain only antibodies can be obtained after immunisation with a specific antigen and in vivo selection for high affinity antibody producing cells. Such cells may be used in a number of procedures depending on application/purpose of use of the antibody.

1) They may be used to produce monoclonal antibody expressing cells by standard techniques.

2) The genes and more importantly the cDNAs coding for these humanised

antibodies (or non humanised antibodies when constant regions from other species are used in the construct) can be cloned as recombinant DNA, either directly from the population of mouse cells isolated after the immunisation or from the monoclonal antibody expressing cells mentioned under 1).

3) A cloned cDNA may be used as a backbone gene to construct an in vitro antibody library. This will also be done by cloning together a V_{HH} gene onto the DJ and a heavy chain region (Cμ, Cα, Cδ, Cγ3 etc. region) of which the C_H1 region has been deleted. However, the advantage of an in vivo selection is lost in this procedure.

The cloned (high affinity) antibodies can be expressed in a number of systems (from micro-organisms to whole animals). In addition any number of protein moieties can be added to the heavy chain antibodies only (e.g. tags to visualise a biological process in vivo or a matrix binding moiety to capture particular antigens). The antibodies can be used for different purposes, i.e. as therapeutics, diagnostics or (laboratory) reagents *in vitro* or *in vivo*.

An advantage of the present invention is that humanised antibodies suitable for a variety of such applications may be produced very rapidly, without the requirement for selection from libraries and scale-up of laboratory procedures. The antibodies of the invention may be used as polyclonal antisera, selected by affinity chromatography, or as monoclonal antibodies as described above.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Claims

1. A transgenic non-human mammal which expresses a heterologous camelid heavy chain locus.

2. A transgenic non-human mammal according to claim 1, wherein said camelid heavy chain locus comprises:

(i) a first locus including a V_{HH} region, a D region and a J region capable of recombining to form a VDJ coding sequence encoding a variable heavy chain polypeptide including a complete antigen binding site; and

(ii) a second locus including at least one exon encoding a constant heavy chain polypeptide

wherein the first and second loci are expressible and their respective coding sequences are capable of recombination to encode a complete heavy polypeptide chain.

3. A transgenic non-human mammal according to claim 1 or claim 2, wherein the coding sequences are from a camelid and another mammal, such as a human.

4. A transgenic non-human mammal according to any preceding claim wherein the V_{HH} region of the first locus comprises at least one V_{HH} exon.

5. A transgenic non-human mammal according to claim 4 wherein the V_{HH} region comprises at least 2, at least 5 or at least 10 V_{HH} exons.

6. A transgenic non-human mammal according to any one of the preceding claims wherein the D region of the first locus comprises at least one D exon of human or camelid origin.

7. A transgenic non-human mammal according to claim 6 wherein the D region comprises at least 2, at least 5 or at least 10 D exons.

8. A transgenic non-human mammal according to any one of the preceding claims wherein the J region of the first locus comprises at least one J exon of human or camelid origin.

9. A transgenic non-human mammal according to claim 8 wherein the J region comprises at least 2, at least 5 or at least 10 J exons.

10. A transgenic non-human mammal according to any one of the preceding claims wherein the exon included in the second locus is one or more exons selected from the group of exons encoding a constant heavy chain ("C_H exons") comprising: Cμ, Cδ, $Cγ_{1-4}$, Cε and $Cα_{1-2}$.

11. A transgenic non-human mammal according to claim 10 wherein the second locus includes a Cμ exon and at least one other C_H exon.

12. A transgenic non-human mammal according to claim 10 wherein the second locus includes a Cμ exon, a Cδ exon and at least one other C_H exon.

13. A transgenic non-human mammal according to claim 10 wherein one or more of the C_H exon(s) in the second locus is modified to render them incapable of expressing the C_H1 portion of their encoded polypeptide.

14. A transgenic non-human mammal according to claim 13 wherein the C_H1 domain of the C_H exon(s) is modified to remove a splice acceptor site 5' of the C_H1 domain.

15. A transgenic non-human mammal according to claim 14 wherein all the C_H exons included in the second locus are modified to render them incapable of expressing their C_H1 domain.

16. A transgenic non-human mammal according to claim 14 wherein the second locus also includes at least one C_H exon including a functional C_H1 domain.

17. A transgenic non-human mammal according to claim 16 wherein the functional C_H1 domain is the C_H1 domain of the Cμ and/or Cδ exon.

18. A transgenic non-human mammal according to any one of the preceding claims wherein the second locus further comprises an immunoglobulin heavy chain LCR.

19. A vector encoding a heavy chain locus according to any one of claims 1-18.

20. A vector according to claim 19 capable of replicating in a host to produce multiple copies of the locus.

21. A vector according to claim 21 capable of inserting the locus into a recipient genome and thus achieving transformation.

22. A method of producing a transgenic animal capable of producing immunoglobulin lacking light polypeptide chains and comprising two heavy polypeptide chains the method comprising inserting into the genome of the animal:

(i) a first locus including a V_{HH} region, a D region and a J region capable of recombining to form a VDJ coding sequence encoding a variable heavy chain polypeptide including a complete antigen binding site; and

(ii) a second locus including at least one exon encoding a constant heavy chain polypeptide;

wherein, upon insertion, the first and second loci are expressible and their respective coding sequences are capable of recombination to encode a complete heavy polypeptide chain.

23. A method of producing single chain antibodies comprising administering an antigen to a transgenic animal according to any one of claims 1-19 or to a transgenic animal produced according to the method of claim 22.

24. An antibody produced according to claim 23.

25. A polyclonal antibody according to claim 24.

26. A monoclonal antibody according to claim 24.

27. A hybridoma capable of producing a monoclonal antibody of claim 26.

28. Use of an antibody according to any one of claims 24 to 27 for diagnostic or therapeutic purposes.

29. Use of a monoclonal antibody according to claim 26 to produce anti-idiotype antibodies.

30. Use of an anti-idiotype antibody produced according to claim 29 in therapy.

31. A pharmaceutical composition comprising an antibody according to any one of claims 24 to 27 and a pharmaceutically acceptable carrier.

32. A single chain antibody producing cell isolated from a transgenic animal according to any one of claims 1-19.

33. A hybridoma derived from the cell of claim 32.

34. An isolated nucleic acid sequence encoding a single chain antibody according to claims 24 to 27..

35. A vector comprising the nucleic acid sequence of claim 34.

36. A host comprising the vector of claim 35.

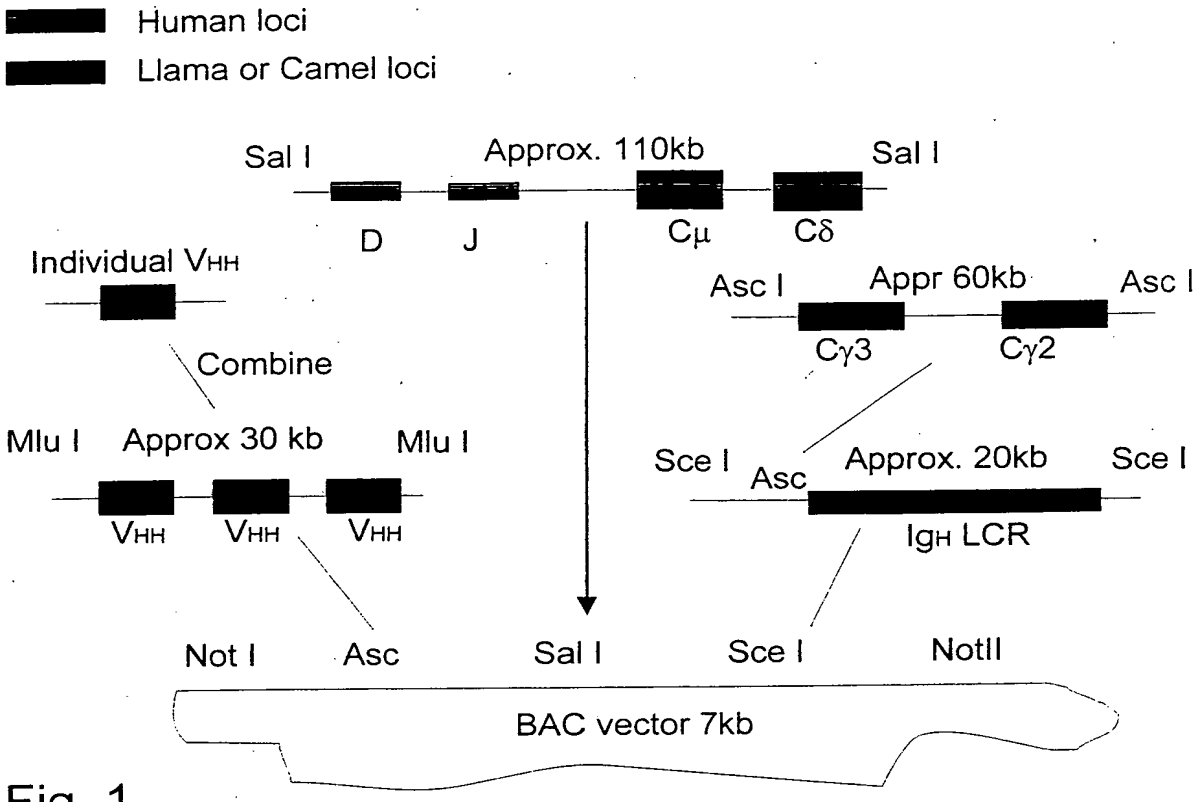
Human loci
Llama or Camel loci
Sal I
Approx. 110kb
Sal I
D
J
Cμ
Cδ
Individual VHH
Asc I
Appr 60kb
Asc I
Cγ3
Cγ2
Combine
Mlu I
Approx 30 kb
Mlu I
VHH
VHH
VHH
Sce I
Asc
Approx. 20kb
Sce I
IgH LCR
Not I
Asc
Sal I
Sce I
NotII
BAC vector 7kb

Fig. 1

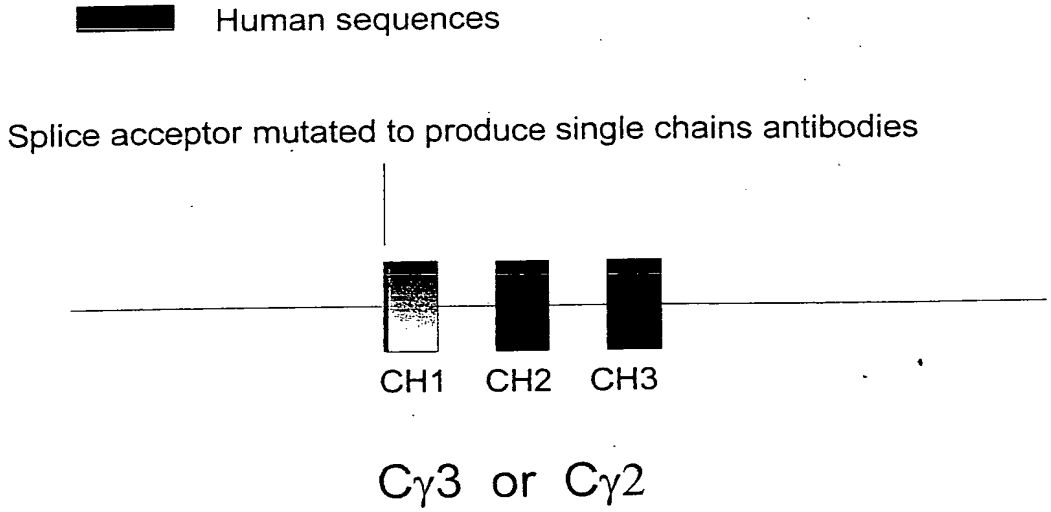
Human sequences
Splice acceptor mutated to produce single chains antibodies
CH1
CH2
CH3
Cγ3 or Cγ2

Fig. 2

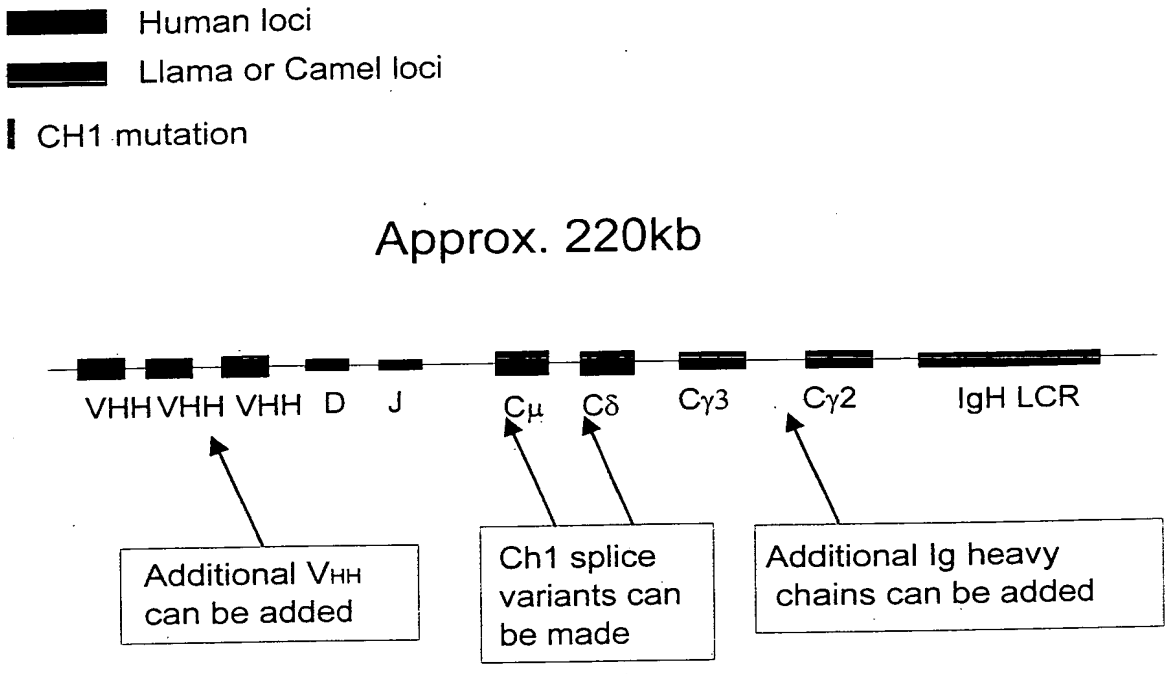
Human loci
Llama or Camel loci
CH1 mutation
Approx. 220kb
VHH VHH VHH D J Cμ Cδ Cγ3 Cγ2 IgH LCR
Additional VHH can be added
Ch1 splice variants can be made
Additional Ig heavy chains can be added

Fig. 3